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(54) Title: IMMUNOTHERAPY FOR AUTOIMMUNE DISEASE			
(57) Abstract A treatment for autoimmune disease based on delivery of antigen by gene gun into the skin, or delivery of antigen-encoding DNA into the skin is described. Delivery of collagen or collagen-coding DNA is useful for treating rheumatoid arthritis, and delivery of myelin basic protein (MBP) or MBP-coding DNA is useful for treating multiple sclerosis. These antigens mitigate cytotoxic responses and elicit antigen desensitization. Other autoimmune diseases can be treated using other antigens.			

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IMMUNOTHERAPY FOR AUTOIMMUNE DISEASE

Technical Field

The present invention relates to the use of immunotherapies for the treatment of autoimmune disorders in general and relates, in particular, to the use of techniques of *in situ* antigen or gene delivery for the treatment of autoimmune diseases.

Background of the Invention

15

Autoimmune Diseases

Autoimmune diseases are characterized by cytotoxic immune responses to epitopes on self antigens natively found in the diseased individual. The immune system of the individual then activates an inflammatory cascade aimed at cells and tissues presenting those specific self antigens. The destruction of the antigen, tissue, cell type, or organ attacked by the individual's own immune system gives rise to the symptoms of the disease.

Clinically significant autoimmune diseases include, for example, rheumatoid arthritis, multiple sclerosis, juvenile-onset diabetes, systemic lupus erythematosus, autoimmune uveoretinitis, autoimmune vasculitis, bullous pemphigus, myasthenia gravis, autoimmune thyroiditis or Hashimoto's disease, Sjogren's syndrome, granulomatous orchitis, autoimmune oophoritis, Crohn's disease, sarcoidosis, rheumatic carditis, ankylosing spondylitis, Grave's disease, and autoimmune thrombocytopenic purpura. See e.g., Paul, W.E. (1993) *Fundamental Immunology*, Third Edition,

Raven Press, New York, Chapter 30, pp. 1033-1097; and Cohen et al. (1994) *Autoimmune Disease Models, A Guidebook*, Academic Press, 1994.

Triggering events for most autoimmune
5 diseases are generally unknown. One theory is that
autoimmune disease may be triggered by infection,
either clinical or sub-clinical, by an infectious
agent which presents the immune system with an epitope
10 that is similar to an epitope natively present on some
cell type in the individual. This theory may explain
the semi-inheritable nature of many autoimmune
diseases since some familial alleles of particular
cell surface markers may be more or less similar to
15 the epitope on the actuating infectious agent, and
therefore more or less likely to be recognized by
antibodies or T-cells that are specific to the
infectious agent.

Conventional therapies for autoimmune
disease tend to focus on symptomatic relief or
20 generalized immune suppression rather than treatment
of the specific underlying immune dysfunction. Such
therapeutic approaches are based on the premise that
if tolerance can be induced to the specific antigen
against which the immune system is acting, progression
25 of the disease should be halted.

Evolving studies of the immune system have
led to a generalization of some immune responses into
two categories, the categories characterized by the
class of helper T-cells activated. These general
30 categories are referred to as Th1 and Th2. Th1 refers
to a cytotoxic T lymphocytic response (a CTL
response), also called a delayed hypersensitivity type
reaction (DTH). This type of response generally
results in destruction of the antigen, cell, tissue,
35 or organ which expresses the antigen. In contrast to
the cytotoxic Th1 type of response, the Th2 T

lymphocytic response generally does not participate in delayed type hypersensitivity or cytotoxic responses. Thus, elimination of cytotoxic responses and development of desensitizing responses are considered
5 to be indicia of development of tolerance to the antigen.

An immune response in either of these two categories tends to down-regulate the immune response in the other category. In this regard, interferon-
10 gamma released by cytotoxic T cells inhibits proliferation of Th2 cells, and interleukin-10 released by Th2 cells inhibits the stimulation of cytotoxic cells by monocytic antigen presenting cells. This mutual inhibition tends to favor the development
15 of only one type of response, cytotoxic or desensitizing, to a given antigen. Because of the mutual inhibition and because an animal's immune system is constantly exposed to self antigens, it has been difficult to intervene in the autoimmune process.

20

Rheumatoid Arthritis

Rheumatoid arthritis is an inflammatory, autoimmune disease affecting multiple systems, but
25 primarily affecting multiple joints. The disease is accompanied by inflammation of the synovial membranes and other joint structures, muscle atrophy, bone erosion and rarefaction, and formation of pannus (fibrovascular inflammatory membrane) on the joint
30 surfaces. Rheumatoid arthritis generally is quite painful and often severely debilitating.

Rheumatoid arthritis is not associated with known infection. The disease is commonly associated with formation of autoantibodies reactive with the
35 subject's own IgG (rheumatoid factor), leading to immunoglobulin aggregates, the role of which in the

disease is unclear. Like many autoimmune diseases, rheumatoid arthritis is more common (~3-fold) in human females than in males. Family studies, including studies of identical and fraternal twins, clearly show
5 that the causation of rheumatoid arthritis is partly genetic and partly non-genetic. The non-genetic component may be either environmental and/or caused by random chance (e.g. somatic mutations, chance biochemical events, stochastic rearrangement of T-cell
10 receptor genes).

Class II genes of the MHC are important in the disease. Approximately 59-70% of patients with clearly diagnosed rheumatoid arthritis have HLA-DR4, compared with ~20-28% of the general population. The
15 most susceptible individuals are heterozygotes with HLA-DR4 (and DQ8) on one copy of chromosome 6 and HLA-DR1 on the other. Recent transgenic mouse studies suggest that HLA-DR and HLA-DQ affect rheumatoid arthritis susceptibility via different mechanisms.

20 One animal model for the study of human rheumatoid arthritis is an autoimmune arthritis induced in rodents. In order to generate disease, Lewis rats or DBA/1 mice, are intradermally injected with type II collagen (CII) in adjuvant (see, e.g.,
25 Stuart et al., *Ann. Rev. Immunol.* 2:199-218 (1984)). This model is referred to as collagen-induced arthritis or CIA.

Prior Studies of Antigen Tolerance in CIA

30 There are reports in which CII has been used to develop tolerance to the antigen to prevent or ameliorate experimental autoimmune arthritis or human rheumatoid arthritis. Whole CII protein or CII-derived peptides have been given, either orally or by
35 injection. For example, Nagler-Anderson et al., *J. Exp. Med.* 170:1999-2010 (1989) reported that

prophylactic feeding of native, but not denatured, bovine CII reduced the incidence of CIA in DBA/1 mice. In contrast, other experimenters have found that the protein can be native or denatured, and peptides are also effective (Khare et al., *J. Immunol.* 155:3653-3659 (1995)).

The importance of 3-dimensional structure in some protocols suggests that antibodies may play a role in the protection in those instances. Alternatively, the denatured protein may somehow be less available for processing for presentation to T cells. The only immunological variable found to be greatly altered in the Nagler-Anderson study was the reduction in CII-specific IgG2b in the successfully protected mice, compared to those that were not pre-treated or were CII-fed but not successfully protected.

Trentham et al., *Science* 261:1727-1730 (1993) conducted a double-blind placebo-controlled clinical trial of 28 chicken CII-fed and 31 placebo-fed patients with rheumatoid arthritis. The authors reported that clinical and lab variables were similar in the collagen and placebo groups at entry. However, despite the stated random allocation to collagen or placebo groups, the placebo group started out significantly worse than the collagen group by many of the assessment criteria used. In an unpublished, larger multi-center clinical trial organized by the same group, oral collagen was effective, but only at the lowest dose.

In feeding-induced tolerance experiments using hen egg lysozyme in mice and guinea pig MBP in rats, Friedman and Weiner (*Proc. Natl. Acad. Sci. USA* 91:6688-6692 (1994)) reported a dose-dependent induction of either anergy (at high antigen doses) or active suppression (at low antigen doses). Cells from

low-dose-treated mice (but not those from mice treated with high antigen doses) were able to down-regulate the *in vitro* proliferative response to other antigens (a phenomenon called "bystander suppression"), not
5 given to the cells, as long as the treatment antigen was present in the same culture. Bystander suppression was induced only at low antigen doses. In contrast, D. Wraith (conference presentation cited in *Immunol. Today* 17:9-12 (1996)) was unable to find
10 differences in the effects of high- versus low-dose tolergen feeding on Th1 and Th2 responses.

In summary, desensitization by oral feeding type II collagen is safe and may be clinically effective, although its effectiveness in rheumatoid
15 arthritis patients has not been clearly demonstrated. Its effectiveness is highly dose-dependent, which would clearly complicate its application in the heterogeneous human population.

Another approach in prior investigations of
20 inducing tolerance in the CIA model involves the injection of collagen or collagen-related peptides into an individual. The CII protein or CII-derived peptides have also been used to treat or prevent CIA and in other autoimmune arthritis models (not
25 collagen-induced).

Like the collagen feeding study, a study by Schoen et al., *J. Immunol.* 128:717-719 (1982), using intravenous injection of CII-coupled spleen cells, required native CII to prevent CIA. Another
30 similarity is that both studies decreased the incidence but not the severity of arthritis. Intravenous CII reduced the anti-CII antibody titers approximately 50%, but did not affect the delayed-type hypersensitivity response to CII. The authors
35 suggested that protection was due to a decrease in a class or subclass of autoantibodies. CI could not be

substituted for CII in the desensitization regimen. Another study (Phadke et al., *Arthritis Rheum.* 27:797-806 (1984)) suppressed CIA via intravenous injection of either native or denatured CII, but native CII was
5 much more effective.

In addition to desensitization regimens, a synthetic modified collagen peptide (designed to competitively inhibit binding of the corresponding native collagen peptide to MHC) reduced the incidence
10 and severity of CIA in mice. For clinical application, this approach is less promising than induction of an anti-inflammatory "Th2-like" response since it likely would require the continued presence of large quantities of the synthetic competitor
15 peptide and is not expected to have a bystander effect.

Similar desensitization procedures have been performed using the 65-kDa heat-shock protein from *Mycobacterium tuberculosis*, or Mt Hsp65. Mt Hsp65 is
20 known to cross-react with a component of articular cartilage, now known to be human Hsp60. This and other heat-shock proteins are up-regulated in the joints of arthritic patients. Rheumatoid arthritis patients have T-cell reactivity to Hsp65, and
25 arthritogenic T cell clones from CFA-treated rats recognize a specific epitope of Hsp65. However, Hsp65 emulsified in oil (in place of whole, killed *Mycobacterium tuberculosis* in oil) did not induce adjuvant arthritis (AA), but instead protected against
30 a subsequent attempt to induce adjuvant arthritis with whole *Mycobacterium tuberculosis*. The 9-amino acid immunodominant epitope was similarly protective.

Most of the reports of arthritis prevention via mycobacterial Hsp65 vaccination have used the
35 adjuvant arthritis model in which Hsp65 is implicated as the major antigen. It is important to determine if

the protection is strictly antigen-specific (as with anergy or clonal deletion) or if bystander suppression occurs (as expected with anti-inflammatory cytokines, for example).

5

Multiple Sclerosis

Multiple sclerosis is a chronic central nervous system disease of considerable medical importance. See Martin et al., Immunological aspects of demyelinating diseases. *Ann. Rev. Immunol.* 10:153-187 (1992). It is the most common demyelinating disorder of the brain and spinal cord. It affects males and females equally, usually beginning between the ages of 20 and 40 years. It can have a subtle onset, with spontaneous remissions and relapses, but its course is often progressive and sometimes relentless.

Pathologically, multiple sclerosis is characterized by localized demyelination of white matter with formation of characteristic plaques. Microscopically, infiltrates of both cytotoxic and helper T cells, as well as macrophages are observed. T cells reactive to myelin basic protein (MBP) and proteolipid protein (PLP) have been found in the cerebrospinal fluid and blood of patients with multiple sclerosis.

The MBP molecule contains multiple epitopes, and those recognized by multiple sclerosis and normal T cells may differ. T cell lines derived from patients with multiple sclerosis recognize an HLA-DR2-restricted epitope in residues 84-106 (Ota et al., *Nature* 346:183-187 (1989)). Additionally, Martin et al., *J. Exp. Med.* 173:19 (1991), identified an epitope in residues 87-106 that was recognized by multiple sclerosis T cells.

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the central nervous system, which, because of its similarities to multiple sclerosis (MS) has often been used as an animal model of that disease (Paterson, P.Y., *Textbook of Immunopathology* (eds Miescher, P.A. and Mueller-Eberhard, H.J.) 179-213 (Grune & Stratton, New York), 1976; Alvord, E.C., Jr. In *Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis* (ed. Alvord, E.C.) 1-511 (Liss, New York, 1984); Steinman, L., *Sci. Am.* 269:106-114 (1993)). The pathology of EAE is characterized by lymphocytic and mononuclear cell inflammation, an increase in blood-brain barrier permeability, and demyelination (Raine et al., *Lab Invest.* 43:150-157 (1980); Paterson et al., *Immunol. Rev.* 55:89-120 (1981)) resulting in partial or complete paralysis and in severe cases death of the affected patient. It is known that neural antigen-specific CD4+ T lymphocytes appear to be the initiators of the response since in vivo depletion of CD4+ T cells inhibits induction of EAE (Waldor et al., *Science* 227:415-417 (1985)) and CD4+ T cell lines and clones can passively transfer disease (Holda et al., *Eur. J. Immunol.* 12:453-455 (1982); Ben-Nun et al., *J. Immunol.* 129:303-308 (1982)).

Autoimmune encephalomyelitis, which occurs in people either as a result of vaccination or following viral infections of the central nervous system (also called post-infectious encephalomyelitis or acute disseminated encephalomyelitis), appears to be analogous to the actively induced form of EAE found in experimental animals in that it is characterized by infiltration of inflammatory cells into the central nervous system, breakdown of the blood brain barrier and demyelination resulting in, inter alia, partial or complete paralysis and in severe cases death

(Anderson, J.R., *Current Topics in Pathology* 76:23-60 (1988); Rabinowitz et al., *J. Neurol. Sci.* 60:393-400 (1983)). Like the animal model, antibodies against the neural myelin basic protein are found in patients
5 who survive this disease (Anderson, J.R., (1988) *supra*; Rabinowitz et al., (1983), *supra*).

The autoreactive T cells that induce organ specific autoimmune diseases, including EAE, generally display a helper T cell type 1 (Th1) phenotype (Miller
10 et al., *Immunol. Today* 15:356 (1994)). The adoptive transfer of myelin-reactive Th1 cells but not Th2 cells can induce EAE and cytokines associated with a Th1 type response are present in the inflammatory lesions of the central nervous system of animals
15 affected by EAE, whereas cytokines associated with a Th2 type are absent from such lesions. Based on these findings, it has been suggested that Th1 cytokines may play a role in the pathology of EAE (Kuchroo et al., *J. Immunol.* 151:3776 (1992); Zamvil et al., *Ann. Rev. Immunol.* 8:579 (1990)).
20

In contrast, regulatory cells involved in suppressing the development of EAE are those associated with a cytokine profile of the Th2 phenotype, and recovery from EAE is associated with
25 the presence of Th2 cells and cytokines in the CNS (Khoury et al., *J. Exp. Med.* 148:3776 (1992); Kennedy et al., *J. Immunol.* 149:2496 (1992)). The latter findings suggest that the preferential induction and activation of Th2 type cells by the appropriate
30 antigen could potentially be useful in the prevention of EAE and other autoimmune diseases caused by Th1 cells.

Diabetes Mellitus

35 Diabetes mellitus is a disorder of glucose metabolism due to a relative or absolute lack of

insulin (reviewed in Atkinson et al., *Sci. Am.* 263:62-71 (1990) and further described in Paul, W.E., *Fundamental Immunology*, 3rd edition, Raven Press, New York, 1993). There are two principal forms of the disease, type I and type II diabetes. Type I diabetes is characterized by the patient's dependency upon exogenous insulin to maintain normal glucose metabolism. Type I diabetes is an autoimmune disease that destroys the insulin-producing beta cells of the islets of Langerhans in the pancreas. The specific lesion, called "insulitis" is characterized by an infiltration of the islets of Langerhans by mononuclear cells, mainly CD8+ T cells, and to a lesser extent by CD4+ T cells (Rossini et al., *Annu. Rev. Immunol.* 3:289-320 (1985)).

Autoantibodies against islet cells are characteristic in type I diabetes, some bind to cytoplasmic constituents, and others bind to proteins on the beta-cell membrane. A notable feature of these anti-islet cell antibodies is that they precede the onset of the disease, and some subsets (such as complement-fixing IgG anti-islet cell antibodies) can predict the disease in susceptible individuals. Antibodies against a cytoplasmic 64 kD beta-cell protein occur in about 80% of newly diagnosed patients, and over 90% of patients have antibodies against particular tryptic fragments of the protein (Christie et al., *J. Exp. Med.* 172:789-794 (1990)). These antibodies may be present for years before the onset of disease, and thus may be predictive markers. Another potential immunogen is a 38-kD beta-cell granule protein. It stimulates *in vitro* proliferation of T cells from diabetics (Roep et al., *Nature* 345:632-634 (1990)). This immune response may provide the link between cytotoxic T-cell responses and type I diabetes

Unfortunately, current therapy for type I diabetes primarily consists of injection of insulin. However, the ability to predict the disease by means of assays for anti-islet cell antibodies, along with
5 the availability of tests for residual beta-cell function, raises the possibility of preventive therapy. This possibility is of substantial interest because in many cases, almost all beta-cells have been destroyed by the time of diagnosis. Clinical trials
10 point to the efficacy of immunosuppressive drugs in type I diabetes (Schwartz, R.P., *New. Engl. J. Med.* 319:649 (1988)), but whether these potent agents should be used in suspected pre-diabetics is debatable. Thus, a more acceptable preventive therapy
15 would be desirable.

Summary of the Invention

The present invention provides an effective method for treating an autoimmune disease. The method
20 is based on the induction of self antigen desensitization in an individual by introduction of the self antigen, or a gene coding therefor, into a cell of the individual. The antigen is selected on the basis of its involvement in the autoimmune
25 process.

Accordingly, in one embodiment, the subject invention is directed to a method for treating or preventing an autoimmune disease in a mammal comprising the steps of:

30 (a) providing a particle coated with an antigen against which an immune response is mounted in the autoimmune disease, wherein the particle is suitable for delivery into a recipient cell, the particle having an average diameter of about 0.5 to
35 about 5 μm and a density sufficient to allow delivery into the recipient cell;

(b) delivering the particle into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic
5 immune response or a desensitizing immune response is induced in the mammal.

In another embodiment, the subject invention is directed to a method for treating or preventing an autoimmune disease in a mammal comprising the steps
10 of:

(a) providing a nucleic acid construct which comprises a coding sequence for an antigen against which an immune response is mounted in the autoimmune disease, operably linked to control elements such that
15 the coding sequence can be transcribed and translated in a recipient cell;

(b) delivering the nucleic acid construct into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number
20 of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

In a related embodiment, the invention is directed to a method for treating or preventing an
25 autoimmune disease in a mammal, wherein the method comprises:

(a) coating a particle with a nucleic acid construct which comprises a coding sequence for an antigen against which an immune response is mounted in
30 the autoimmune disease, operably linked to control elements such that the coding sequence can be transcribed and translated in a recipient cell, wherein the particle has an average diameter of about 0.5 to about 5 μm and a density sufficient to allow
35 delivery into the recipient cell;

(b) delivering the particle into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic
5 immune response or a desensitizing immune response is induced in the mammal.

In particularly preferred embodiments, the cytotoxic immune response is characterized by the secretion from a T-lymphocyte of one or more mediators
10 selected from the group consisting of interleukin-2, interferon-gamma and tumor necrosis factor. The desensitizing immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-4,
15 interleukin-5, interleukin-6, and interleukin-10. In one related embodiment, the desensitizing immune response is characterized by augmented production of one or more mediators selected from the group consisting of interleukin-4, interleukin-10, and,
20 surprisingly, interferon-gamma.

In additional embodiments, the autoimmune disease is rheumatoid arthritis and the antigen is selected from the group consisting of collagen, the *Mycobacterium tuberculosis* heat shock protein Mt
25 Hsp65, and epitopes thereof.

In yet further embodiments, the autoimmune disease is multiple sclerosis and the antigen is selected from the group consisting of myelin basic protein, myelin oligodendrocyte glycoprotein,
30 proteolipid protein, and epitopes thereof.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Drawings

Fig. 1 depicts the results of an experiment where type II collagen (CII) was delivered to mice.

Fig. 2 shows IgG subclass responses in CII-challenged mice.

Fig. 3 shows IgG subclass responses in Hsp65-DNA-pretreated, CII-challenged mice.

Fig. 4 shows the protocol for immunizing rats for treatment of experimental autoimmune encephalomyelitis (EAE).

Detailed Description of the Preferred Embodiments

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular antigens, antigen-coding nucleotide sequences, or the specifically enumerated diseases. It is also understood that different applications of the disclosed methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more such agents, reference to "a particle" includes reference to mixtures of two or more particles, and the like.

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the

art to which the invention pertains. The following terms are intended to be defined as indicated below.

As used herein, the term "autoimmune disease" means a set of sustained organ-specific or systemic clinical symptoms and signs associated with altered immune homeostasis that is manifested by qualitative and/or quantitative defects of expressed autoimmune repertoires (*Autoimmunity Physiology and Disease*, Coutinho and Kazatchkine, eds, Wiley-Liss, 1993, Chapter 27, page 433).

An "antigen" refers to any agent, generally a macromolecule, which can elicit an immunological response in an individual. The immunological response may be of B- and/or T-lymphocytic cells. The term may be used to refer to an individual macromolecule or to a homogeneous or heterogeneous population of antigenic macromolecules. As used herein, "antigen" is used to refer to a protein molecule or portion thereof which contains one or more epitopes.

The term "epitope" generally refers to the site on an antigen to which a specific antibody molecule binds. The identification of epitopes which are able to elicit an antibody response is readily accomplished using techniques well known in the art. See, e.g., Geysen et al. *Proc. Natl. Acad. Sci. USA* (1984) 81:3998-4002 (general method of rapidly synthesizing peptides to determine the location of immunogenic epitopes in a given antigen); U.S. Patent No. 4,708,871 (procedures for identifying and chemically synthesizing epitopes of antigens); and Geysen et al., *Molecular Immunology* (1986) 23:709-715 (technique for identifying peptides with high affinity for a given antibody). T-cell epitopes are generally those features of a peptide structure capable of inducing a T-cell response. In this regard, it is accepted in the art that T-cell epitopes comprise

linear peptide determinants that assume extended conformations within the peptide-binding cleft of MHC molecules, (Unanue et al., *Science* (1987) 236:551-557). As used here, an epitope is generally a peptide
5 having about 3-5, preferably 5-10 or more amino acid residues. Particular epitopes useful in the practice of the invention include, but are not limited to, the 9 amino acid immunodominant epitope of Hsp65 and the epitope present in residues 84-106 of the MBP
10 molecule.

The term "self antigen," which is used interchangeably herein with the term "autoantigen," means an antigen, or a molecule capable of being recognized during an immune response, that is normally
15 part of the individual. This is in contrast with antigens which are foreign, or exogenous, which are not normally part of the individual's milieu. Each autoimmune disease is characterized by an immune response directed at a self antigen. Normally, there
20 are no active immune responses to self antigens, and no symptoms appear. With the development of an immune response to a self antigen, autoimmune diseases may appear. Autoimmune diseases present clinically with different symptoms depending upon the specific self
25 antigen against which an immune response is raised. This immune response results in the destruction of the structure containing the self antigen, and it is the loss of that structure with concurrent loss of that structure's normal function which results in symptoms
30 of autoimmune disease.

As used herein, the term "anergy" means a reversible antiproliferative state which results in decreased responsiveness of an immune cell or cells to an antigen.

35 The term "antigen desensitization" refers to the process of decreasing an immune response by

delivering to the cell or animal, over a period of time, the antigen against which an immune response is mounted. With repeated exposure of the immune cells to the antigen, a decrease in the cytotoxic response is seen. Such desensitization includes, but is not limited to, a switch from a "Th1" to a "Th2" response. Antigen desensitization may result in the cessation of the autoimmune process, and may ultimately result in the replenishment of the individual's self antigen, and the associated structures.

The term "Th1" means that response of T-lymphocytes to antigen characterized by, but not limited to, T-lymphocyte secretion of one or more of the following mediators: interleukin-2 (IL-2); interferon-gamma (IFN-gamma); and tumor necrosis factor-beta (TNF-beta). The lymphokines and cells activated during a Th1 response are primarily mediators of delayed hypersensitivity type (DTH) or cytotoxic reactions. It is understood that other mediators may be released without changing the meaning of "Th1."

The term "Th2" means that response of T-lymphocytes to antigen characterized by, but not limited to, T-lymphocyte secretion of one or more of the following mediators: interleukin-4 (IL-4); interleukin-5 (IL-5); interleukin-6 (IL-6); and interleukin-10 (IL-10). The lymphokines produced by Th2 cells are primarily mediators of helper T-cell function for B-cell antibody production, and are not generally part of the delayed hypersensitivity type response (DTH) or of the cytotoxic response. Th2 responses are generally desensitizing to the antigen. It is understood that other mediators may be released without changing the meaning of "Th2."

By "vector" is meant any genetic element, such as a plasmid, phage, transposon, cosmid,

chromosome, virus, virion, etc., which is generally capable of replication when associated with the proper control elements and present in an appropriate host cell.

5 "Gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons
10 (e.g., episomes), integration of transferred genetic material into the genomic DNA of host cells, or eventual loss of the DNA through degradative processes.

A "nucleotide sequence" or a "nucleic acid
15 molecule" refers to DNA and RNA sequences. The term captures molecules that include any of the known base analogues of DNA and RNA.

A "coding sequence" or a sequence which "encodes" a particular polypeptide, is a nucleic acid
20 sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are conventionally determined by a
25 start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eukaryotic mRNA, genomic DNA sequences from procaryotic or eukaryotic DNA, and even
30 synthetic DNA sequences. In eukaryotic DNA, a transcription termination sequence will usually be located 3' to the coding sequence.

The term DNA "control sequences" refers collectively to promoter sequences, polyadenylation
35 signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal

ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences
5 need always be present so long as the selected gene is capable of being transcribed and translated in an appropriate recipient cell.

"Operably linked" refers to an arrangement of elements wherein the components so described are
10 configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as
15 they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding
20 sequence.

B. General Methods

Before describing the present invention in detail, it is to be understood that this invention is
25 not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be
30 limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

35 As explained above, the present invention allows for the treatment and/or prevention of a wide

variety of autoimmune diseases including, but not limited to, rheumatoid arthritis, multiple sclerosis, juvenile-onset diabetes, systemic lupus erythematosus, autoimmune uveoretinitis, autoimmune vasculitis, 5 bullous pemphigus, myasthenia gravis, autoimmune thyroiditis or Hashimoto's disease, Sjogren's syndrome, granulomatous orchitis, autoimmune oophoritis, Crohn's disease, sarcoidosis, rheumatic carditis, ankylosing spondylitis, Grave's disease, and 10 autoimmune thrombocytopenic purpura. The method utilizes autoantigen desensitization carried out using, for example, particle-mediated delivery techniques (e.g., gene gun delivery). A wide variety of nucleic acid molecules or peptide antigens can be 15 delivered using the methods of the invention. The particular molecule selected depends on the autoimmune disease being treated, for example, if the autoimmune disease to be treated is rheumatoid arthritis, the autoantigen can be selected from the group of 20 collagen, the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, epitopes thereof capable of eliciting the appropriate immune response, and nucleic acid molecules encoding the same. If the autoimmune disease is multiple sclerosis, the autoantigen can be 25 selected from the group of myelin basic protein, myelin oligodendrocyte protein, proteolipid protein, epitopes thereof, and nucleic acid molecules encoding the same, as well as coding sequences encoding the entire cDNA library of human oligodendrocytes or an 30 oligodendrocyte gene library.

Generally, nucleic acid molecules used in the subject methods contain coding regions with suitable control sequences and, optionally, ancillary therapeutic nucleotide sequences. The nucleic acid 35 molecules are prepared in the form of vectors which include the necessary elements to direct transcription

and translation in a target cell. If expression is desired using the host's enzymes (such as by the use of endogenous RNA polymerase), the gene or genes will be present in the vectors operatively linked to control sequences recognized by the particular host, or even particular cells within the host. Thus, eukaryotic control elements will be present for expression in mammalian hosts. Such sequences are known in the art and are discussed more fully below.

10 In order to augment anti-inflammatory responses, the above-described autoantigens can be administered in conjunction with ancillary substances, such as pharmacological agents, adjuvants, cytokines, or in conjunction with delivery of vectors encoding cytokines. Furthermore, to avoid the possibility of eliciting unwanted anti-self cytokine responses when using cytokine codelivery, chemical immunomodulatory agents such as the active form of vitamin D3 can also be used. In this regard, 1,25-dihydroxy vitamin D3 has been shown to exert an adjuvant effect via intramuscular DNA immunization.

Ancillary nucleic acid sequences coding for peptides known to stimulate, modify, or modulate a host's immune response, can be coadministered with the above-described antigens. Thus, genes encoding one or more of the various cytokines (or functional fragments thereof), such as the interleukins, interferons, and colony stimulating factors, will find use with the instant invention. The gene sequences for a number of these substances are known. For example, genes encoding IL-4 and IL-10 can be codelivered with autoantigen preparations. Further, the inventors have discovered that autoantigen delivery to the epidermis elicits an autoantigen-specific anti-inflammatory immune response that is characterized by suppression of IgG2 antibody response, T cell proliferation and

IL-2 production, coupled with augmentation of IL-4, IL-10 and interferon gamma (IFN- γ) production. The suppression of IgG2 and IL-2 production, coupled with augmented production of IL-4 and IL-10 is reminiscent of a Th1 to Th2 shift. However, the corresponding augmentation of IFN- γ production is a surprising and unexpected event in an anti-inflammatory response. Elevated IFN- γ responses may thus play a role in the protective mechanism, rather than contributing to autoimmune disease as previously believed. In this regard, recent reports have demonstrated that IFN- γ plays an important down-regulatory role in both the induction and effector phases of myelin oligodendrocyte glycoprotein-induced EAE in mice. Willenborg et al., (1996) *J. Immunol.* 157:3223-3227. Thus, in one embodiment of the invention, delivery of an autoantigen is coupled with codelivery of one or more of the following immunological response modifiers: IL-4; IL-10; and IFN- γ .

In addition, ancillary agents can be used to enhance desensitization, such as where the cytotoxic response is pharmacologically suppressed. In this regard, CIA and AA in rodents, and RA in humans, each appear to be mediated by cytotoxic immune responses. This observation is supported by data showing that both anti-IL-2 receptor monoclonal antibodies and IL-4 can suppress CIA in mice, while IL-12 and interferon-gamma exacerbate disease. Thus, in another embodiment of the invention, autoantigen-specific TH1 immunity can be transiently suppressed using suitable pharmacological agents such as IL-4, anti-IL-2 receptor antibody molecules, anti-TNF-alpha antibodies, and anti-CD4 antibody molecules.

Other approaches to systemically modulating inflammatory cytotoxic immune responses during desensitization to autoantigens is to pretreat and/or

code deliver a vector encoding a known desensitizing antigen such as HIV-1 gp120. In this manner, systemic induction of desensitization may transiently ameliorate active disease symptoms since HIV-1 infection has been reported to have similar effects in human rheumatoid arthritis patients. Alternatively, other means of systemically suppressing inflammatory responses prior to or during active autoantigen delivery is to administer anti-inflammatory compounds such as prednisone or methotrexate. These compounds are currently used to suppress rheumatoid arthritis and other inflammatory diseases in humans, and could sufficiently alter the systemic cytokine balance to enable elicitation of the desired anti-inflammatory effects via autoantigen treatment. Furthermore, in order to take advantage of a bystander effect in the treatment of RA, vectors encoding other articular cartilage antigens, such as the cartilage proteoglycan core proteins, aggrecan and lumican, can be code delivered with RA-specific autoantigens to help decrease cytotoxic responses. In this way, induction of an immune response by T lymphocytes recognizing new self antigens in local sites of inflammation results in the local production of anti-inflammatory cytokines, and ameliorates pathology and symptoms of disease. Expression vectors encoding such articular cartilage antigens can be constructed using, for example, cDNA sequences of human and other vertebrate proteoglycan core protein genes, which are readily available.

Modes of carrying out the invention are described more fully below.

Isolation of Genes and Construction of Vectors

Nucleotide sequences selected for use in the present invention can be derived from known sources,

for example, by isolating the same from cells containing a desired gene or nucleotide sequence using standard techniques. Similarly, the nucleotide sequences can be generated synthetically using
5 standard modes of polynucleotide synthesis that are well known in the art. See, e.g., Edge et al., *Nature* 292:756 (1981); Nambair et al., *Science* 223:1299 (1984); Jay et al., *J. Biol. Chem.* 259:6311 (1984). Generally, synthetic oligonucleotides can be prepared
10 by either the phosphotriester method as described by Edge et al. (*supra*) and Duckworth et al., *Nucleic Acids Res.* 9:1691 (1981), or the phosphoramidite method as described by Beaucage et al., *Tet. Letts.* 22:1859 (1981), and Matteucci et al., *J. Am. Chem.*
15 *Soc.* 103:3185 (1981). Synthetic oligonucleotides can also be prepared using commercially available automated oligonucleotide synthesizers. The nucleotide sequences can thus be designed with appropriate codons for a particular amino acid
20 sequence. In general, one will select preferred codons for expression in the intended host. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g.,
25 Edge et al. (*supra*); Nambair et al. (*supra*) and Jay et al. (*supra*).

Another method for obtaining nucleic acid sequences for use herein is by recombinant means. Thus, a desired nucleotide sequence can be excised
30 from a plasmid carrying the same using standard restriction enzymes and procedures. Site specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the
35 particulars of which are specified by manufacturers of commercially available restriction enzymes. If

d sired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques.

5 Restriction cleaved fragments may be blunt ended by treating with the large fragment of *E. coli* DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using standard techniques. The Klenow fragment fills in at 5' single-stranded overhangs but digests protruding 3'
10 single strands, even though the four dNTPs are present. If desired, selective repair can be performed by supplying only one, or several, selected dNTPs within the limitations dictated by the nature of the overhang. After Klenow treatment, the mixture can
15 be extracted with e.g. phenol/chloroform, and ethanol precipitated. Treatment under appropriate conditions with S1 nuclease or BAL-31 results in hydrolysis of any single-stranded portion.

Yet another convenient method for isolating
20 specific nucleic acid molecules is by the polymerase chain reaction (PCR). Mullis et al., *Methods Enzymol.* 155:335-350 (1987). This technique uses DNA polymerase, usually a thermostable DNA polymerase, to replicate a desired region of DNA. The region of DNA
25 to be replicated is identified by oligonucleotides of specified sequence complementary to opposite ends and opposite strands of the desired DNA to prime the replication reaction. The product of the first round of replication is itself a template for subsequent
30 replication, thus repeated successive cycles of replication result in geometric amplification of the DNA fragment delimited by the primer pair used. This method also allows for the facile addition of nucleotide sequences onto the ends of the DNA product
35 by incorporating these added sequences onto the oligonucleotide primers (see, e.g., *PCR Protocols*, A

Guide to Methods and Applications, Innis et al (eds) Harcourt Brace Jovanovich Publishers, NY (1994)).

Once coding sequences for desired proteins have been prepared or isolated, such sequences can be
5 cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Ligations to other sequences are performed using standard procedures,
10 known in the art.

Selected nucleotide sequences can be placed under the control of regulatory sequences such as a promoter or ribosome binding site (collectively referred to herein as "control" elements), so that the
15 sequence encoding the desired protein is transcribed into RNA in the host tissue transformed by a vector containing this expression construct.

The choice of control elements will depend on the host being treated and the type of preparation used. Thus, if the host's endogenous transcription and translation machinery will be used to express the
20 proteins, control elements compatible with the particular host will be utilized. In this regard, several promoters for use in mammalian systems are known in the art and include, but are not limited to,
25 promoters derived from SV40, CMV, HSV, RSV, MMTV, among others.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for
30 regulation of the expression of protein sequences encoded by the delivered nucleotide sequences. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a coding sequence to be turned on or off
35 in response to a chemical or physical stimulus, including the presence of a regulatory compound.

Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate control and, optionally, regulatory sequences such that the positioning and orientation of the coding sequence with respect to the control sequences allows the coding sequence to be transcribed under the "control" of the control sequences (i.e., RNA polymerase, which binds to the DNA molecule at the control sequences, transcribes the coding sequence). Modification of the sequences encoding the particular protein of interest may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it is attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

25 Preparation of Peptide Antigens

Peptide antigens can be produced using a variety of methods known to those skilled in the art. In particular, the antigens can be isolated directly from native sources, using standard purification techniques. Alternatively, the antigens can be recombinantly produced using expression systems as described above and purified using known techniques. The peptide antigens can also be synthesized, based on described amino acid sequences or amino acid sequences derived from the DNA sequence of a nucleic acid molecule of interest, via chemical polymer syntheses

- such as solid phase peptide synthesis. Such methods are known to those skilled in the art. See, e.g., J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology*, supra, Vol. 1, for classical solution synthesis.
- 15 Administration of Nucleic Acid Preparations
 Methods for delivering nucleic acid preparations are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. A number of viral based systems have been developed for transfer into mammalian cells. For example, retroviral systems have been described (U.S. Patent No. 5,219,740; Miller et al., *BioTechniques* 7:980-990 (1989); Miller, A.D., *Human Gene Therapy* 1:5-14 (1990); Scarpa et al., *Virology* 180:849-852 (1991); Burns et al., *Proc. Natl. Acad. Sci. USA* 90:8033-8037 (1993); and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* 3:102-109 (1993). A number of adenovirus vectors have also been described, see e.g., Haj-Ahmad et al., *J. Virol.* 57:267-274 (1986); Bett et al., *J. Virol.* 67:5911-5921 (1993); Mittereder et al., *Human Gene Therapy* 5:717-729 (1994); Seth et al., *J. Virol.* 68:933-940 (1994); Barr et al., *Gene Therapy* 1:51-58 (1994); Berkner, K.L., *BioTechniques* 6:616-629 (1988); and Rich et al., *Human Gene Therapy* 4:461-476 (1993).
- 35 Adeno-associated virus (AAV) vector systems have also been developed for nucleic acid delivery. AAV vectors

can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* 8:3988-3996 (1988); Vincent et al., *Vaccines 90* (Cold Spring Harbor Laboratory Press) (1990); Carter, B.J., *Current Opinion in Biotechnology* 3:533-539 (1992); Muzyczka, N., *Current Topics in Microbiol. and Immunol.* 158:97-129 (1992); Kotin, R.M., *Human Gene Therapy* 5:793-801 (1994); Shelling et al., *Gene Therapy* 1:165-169 (1994); and Zhou et al., *J. Exp. Med.* 179:1867-1875 (1994).

The nucleic acid molecule of interest can also be delivered without a viral vector. For example, the molecule can be packaged in liposomes prior to delivery to the subject. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug et al., *Biochim. Biophys. Acta.* 1097:1-17 (1991); Straubinger et al., in *Methods of Enzymology*, Vol. 101, pp. 512-527 (1983).

The nucleic acid preparations of the present invention may also be encapsulated, adsorbed to, or associated with, particulate carriers for delivery to suitable recipient cells. Exemplary particulate systems and polymers include, for example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* 5:163-187 (1990), for a review of delivery systems useful for transfer of nucleic acid molecules.

Preparation of Coated Carrier Particles

In preferred embodiments of the invention, the autoantigen preparations are administered using particle mediated delivery techniques. Thus, once
5 prepared and suitably purified, the above-described nucleic acid molecules and peptide antigens can be coated onto carrier particles using a variety of techniques known in the art. Carrier particles are selected from materials which have a suitable density
10 in the range of particle sizes typically used for intracellular delivery from a gene gun device. The optimum carrier particle size will, of course, depend on the diameter of the target cells.

For the purposes of the invention, tungsten,
15 gold, platinum and iridium carrier particles can be used. Tungsten and gold particles are preferred. Tungsten particles are readily available in average sizes of 0.5 to 2.0 μm in diameter. Although such particles have optimal density for use in particle
20 acceleration delivery methods, and allow highly efficient coating with DNA, tungsten may potentially be toxic to certain cell types. Gold particles or microcrystalline gold (e.g., gold powder A1570, available from Engelhard Corp., East Newark, NJ) will
25 also find use with the present methods. Gold particles provide uniformity in size (available from Alpha Chemicals in particle sizes of 1-3 μm , or available from Degussa, South Plainfield, NJ in a particle size of 0.95 μm) and reduced toxicity.
30 Microcrystalline gold provides a diverse particle size distribution, typically in the range of 0.5-5 μm . However, the irregular surface area of microcrystalline gold provides for highly efficient coating with DNAs.

35 A number of methods are known and have been described for coating or precipitating DNA or RNA onto

gold or tungsten particles. Most such methods generally combine a predetermined amount of gold or tungsten with plasmid DNA, CaCl_2 , and spermidine. The resulting solution is vortexed continually during the coating procedure to ensure uniformity of the reaction mixture. After precipitation of the DNA, the coated particles can be transferred to suitable membranes and allowed to dry prior to use, coated onto surfaces of a sample module or cassette, or loaded into a delivery cassette for use in particular gene gun instruments.

Peptide or protein antigens can also be coated onto suitable carrier particles, e.g., gold or tungsten. For example, peptides can be attached to the carrier particle by simply mixing the two components in an empirically determined ratio, by ammonium sulfate precipitation or other solvent precipitation methods familiar to those skilled in the art, or by chemical coupling of the peptide to the carrier particle. The coupling of L-cysteine residues to gold has been previously described (Brown et al., *Chemical Society Reviews* 9:271-311 (1980)). Other methods include, for example, dissolving the peptide antigen in absolute ethanol, water, or an alcohol/water mixture, adding the solution to a quantity of carrier particles, and then drying the mixture under a stream of air or nitrogen gas while vortexing. Alternatively, the peptide antigens can be dried onto carrier particles by centrifugation under vacuum. Once dried, the coated particles can be resuspended in a suitable solvent (e.g., ethyl acetate or acetone), and triturated (e.g., by sonication) to provide a substantially uniform suspension.

Administration of Coated Particles

Following their formation, carrier particles coated with either nucleic acid preparations, or

peptide or protein antigen preparations, are delivered to mammalian tissue using particle-mediated delivery techniques.

Various particle acceleration devices
5 suitable for particle-mediated delivery are known in the art, and are all suited for use in the practice of the invention. Current device designs employ an explosive, electric or gaseous discharge to propel the coated carrier particles toward target cells. The
10 coated carrier particles can themselves be releasably attached to a movable carrier sheet, or removably attached to a surface along which a gas stream passes, lifting the particles from the surface and accelerating them toward the target. An example of a
15 gaseous discharge device is described in U.S. Patent No. 5,204,253. An explosive-type device is described in U.S. Patent No. 4,945,050. One example of an electric discharge-type particle acceleration apparatus is the ACCELL[®] instrument manufactured by
20 Auragen, Inc., Madison, WI, which instrument is described in U.S. Patent No. 5,120,657. Another electric discharge apparatus suitable for use herein is described in U.S. Patent No. 5,149,655.

The coated particles are administered to the
25 subject to be treated in a manner compatible with the dosage formulation, and in an amount that will be prophylactically and/or therapeutically effective. The amount of the composition to be delivered which, in the case of nucleic acid molecules is generally in
30 the range of from 0.001 to 10.0 μ g, more preferably 0.01 to 10.0 μ g of nucleic acid molecule per dose, and in the case of peptide or protein molecules is 1 μ g to 1 mg, more preferably 1 to 50 μ g of peptide, depends on the subject to be treated. The exact amount
35 necessary will vary depending on the age and general condition of the individual to be treated, the

severity of the autoimmune condition being treated and the particular nucleotide sequence or peptide antigens selected, the site of administration, as well as other factors. An appropriate effective amount can be readily determined by one of skill in the art upon reading the instant specification.

Thus, a "therapeutically effective amount" of the antigens, or nucleic acids coding therefor, will be sufficient to bring about treatment or prevention of autoimmune disease or condition symptoms, and will fall in a relatively broad range that can be determined through routine trials.

The coated particles are delivered to suitable target cells, most preferably to cells of the skin, such as the epidermis. In this regard, it has been found that the introduction of autoantigen into the epidermis of a mammal by gene gun can be performed in such a way so as to induce desensitization and decrease in cytotoxic T-cell responses. It may alternatively be desired to deliver the particles to mucosal tissue cells, in order to bring about mucosal, humoral and cellular immune responses in the treated subject.

Delivery of the particles is timed and repeated in such a manner so as to facilitate the suppression of cytotoxic immune responses and facilitate the creation of a desensitizing-type response which will, in turn, inhibit the arousal of a cytotoxic inflammatory immune response. One way to elicit such a desensitizing response in the practice of the invention is to deliver antigen to a subject over a relatively prolonged period of time, for example over a period of several weeks. Such delivery may result in T-cell anergy, a state of reversible non-proliferation. In this condition, cytotoxic T

cells do not proliferate and the cytotoxic effects of autoimmune diseases are minimal.

On the other hand, multiple and frequent deliveries of self antigen-encoding expression vectors via gene gun delivery to the epidermis generally provide transient bursts of localized antigen production that result in the induction of Th2-like responses characterized by a preponderance of IgG1 antibodies, diminished interferon-gamma production, suppressed CTL activity, and elevated IL-4 production. Thus, desensitization to antigens can be brought about using repeated gene gun delivery of antigens directly into the epidermis.

Thus, in one embodiment, a method for the treatment of rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases is provided which involves an active, rather than passive immunotherapeutic strategy. This approach is based on the ability of epidermal antigen delivery to desensitize the immune system to the self antigen, and thereby eliminate the pro-inflammatory cytokine milieu at its source. As stated above, an example of this strategy involves the use of oral tolerance to anergize or actively suppress pro-inflammatory cytotoxic responses. These studies, and those involving the parenteral administration of self or "self-like" antigens in the rodent adjuvant arthritis and collagen-induced arthritis models support the concept of the "bystander" effect as being potentially useful in the treatment of autoimmune disease, particularly in the treatment of human rheumatoid arthritis.

While it is easy to envision how prior collagen and mycobacterial heat shock protein immunizations (Hsp65) can protect against CIA and AA, respectively, evidence for the bystander effect has

come from studies showing that oral collagen administration can suppress AA induction, and that immunization with Hsp65 can suppress arthritis induced via lipoidal amine administration.

5 Because of the apparent bystander phenomenon, strategies based on active immunotherapy do not require that one know the identity of the specific self antigen(s) responsible for disease induction. Continued maintenance of a desensitizing
10 immune response by the judicious administration of appropriate antigens that participate in the bystander effect can keep the disease process in check for an indefinite period of time. Support for such an approach can be found in the observation of partial
15 remission of rheumatoid arthritis in certain patients during pregnancy and early HIV infection.

 Unlike oral tolerance studies in mice and rats, where only partial protection against subsequent autoimmune disease development has been observed, it
20 has been found by the inventors herein that using particle-mediated techniques to deliver type II collagen (as protein), or a mycobacterial heat shock protein gene, results in 100% protection against disease development in a murine CIA model. These
25 results demonstrate that gene gun delivery provides for the heretofore unattainable suppression of antigen-specific pro-inflammatory immune responses.

 Additionally, the present inventors have discovered that treatment of rats which are
30 susceptible to the development of acute encephalomyelitis, via delivery of DNA encoding the gene for myelin basic protein (a major constituent of the myelin sheet surrounding neurons in the central nervous system), protects animals against developing
35 encephalomyelitis. Furthermore, immunization by the nucleic acid-based methods described herein protects

such animals from autoimmune disease of the central nervous system by inducing a switch in the type of immune response which they exhibit after antigen challenge with myelin basic protein (Paterson, P.Y.,
5 *Textbook of Immunopathology* (eds Miescher, P.A. and Meuller-Eberhard, H.J.) 179-213 (Grune & Stratton, New York, 1976).

In contrast to the results seen in animals treated with DNA coding for myelin basic protein,
10 control animals receiving plasmid DNAs not containing the gene for myelin basic protein were not protected from developing EAE. While it is not intended that the present invention should be restricted in any way by a theoretical explanation of the mechanism of
15 action of the efficacy, it is believed that such treatment may exert its beneficial effects by desensitizing the animal and decreasing the cytotoxic type of immune response.

20 C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present
25 invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

30

Example 1

Prevention of Collagen Induced Arthritis with Type II Collagen

In the first attempt to determine if gene
35 gun treatment can elicit protection against the

development of collagen-induced arthritis (CIA), an experiment was designed to determine the following:

(1) whether intradermal injection of bovine type II collagen (CII) protein could elicit CIA in
5 DBA/1 mice;

(2) whether gene gun-mediated delivery of CII to the epidermis would not elicit a similar disease on its own; and

(3) whether gene gun-mediated delivery of
10 CII to the epidermis could elicit CII-specific Th2-like immune responses that might protect against CIA development.

Bovine CII (Sigma, St. Louis) was coated onto 0.95 μ m gold particles via ammonium sulfate
15 precipitation. CII-coated gold particles were washed with ethanol and loaded into individual gene gun shots as previously described (Fuller et al., *supra*). Each shot contained approximately 5 μ g CII protein and 0.5 mg of gold. Epidermal delivery of protein-coated gold
20 particles was performed using the hand-held, helium driven Accell gene delivery system (PCT pat. appl. WO 95/19799). Twenty 6 week old female DBA/1 mice each received single CII gene gun shots to the abdominal epidermis on days 0, 14 and 35 after fur removal by
25 clipping. Half of these animals, and 9 additional naive mice, received subcutaneous injections of 200 μ g CII protein in Freund's complete adjuvant, containing 100 μ g *M. tuberculosis*, on day 28. Incidence of arthritis in each group was plotted. Disease was
30 scored as described by Khare et al., *J. Immunol.* 155:3653-3659 (1995), in which mice were monitored for redness or swelling in paws or toes, severe swelling and joint deformity, and joint ankylosis.

Figure 1 shows the incidence of arthritic
35 disease in these three groups of animals as a function of time. While subcutaneous CII injections in

complete Freund's adjuvant induced arthritic disease in 8 of 9 animals that received the CII/Freund's injections only (■), no animals in the groups given CII by gene gun (●), or CII/Freund's by gene gun (Δ),
5 exhibited any symptoms of arthritis. These data demonstrate that gene gun-mediated administration of CII protein to the epidermis not only failed to elicit CIA on its own, but completely protected against the development of CIA following CII/Freund's challenge.

10 The dramatic protection against the development of any CIA disease symptoms via the delivery of three gene gun-mediated CII doses (2 before and 1 after challenge) suggests that the gene gun-treated animals reacted immunologically to the
15 epidermal delivery of CII protein. To determine if measurable differences could be detected, bovine CII-specific IgG1, IgG2a, and IgG2b responses were measured in all animals six weeks after challenge (day 70); these data are shown in Figure 2. Figure 2 plots
20 the IgG subclass responses in CII-challenged mice. Serum samples from mice immunized as described above were collected on day 70 (42 days after subcutaneous CII/Freund's challenge). CII-specific IgG subclass responses were measured by standard ELISA techniques
25 using bovine CII adsorbed to ELISA plates as the immobilized phase.

The animals in the gene gun-only group exhibited no detectable CII-specific IgG activity, but low IgM titers were seen. This was surprising in view
30 of the relatively strong responses that gene gun immunizations normally elicit (using plasmid DNA vectors), and could be related to the fact that gene gun delivery is largely an intracellular process.

In contrast, the two groups of animals
35 challenged with CII/Freund's (delivered via gene gun or subcutaneously) exhibited significant CII-specific IgG

activity after challenge, but differed in the relative proportions of IgG subclasses, depending on whether or not they received prior gene gun CII treatments. Arthritic animals challenged with CII/Freund's exhibited high levels of CII-specific IgG1, IgG2a, and IgG2b. However, the gene gun-protected animals that failed to develop disease in response to CII/Freund's challenge exhibited a preponderance of CII-specific IgG1, and reduced levels of IgG2a, and IgG2b responses.

These findings are consistent with the conclusion that epidermal gene gun immunizations desensitize animals toward autoantigens, as well as with the hypothesis that such desensitization may be associated with protection in the rodent CIA and AA models of human rheumatoid arthritis.

Example 2

Gene Gun-Mediated Delivery of a Gene Encoding Hsp65 from *M. tuberculosis*

A further experiment was carried out to assess the efficacy of a gene gun-mediated DNA immunization approach to prevention of autoimmune disease. Briefly, two successive Hsp65 DNA treatments to the epidermis, three weeks apart, protected 100% of mice from CIA development after CII/Freund's challenge. 77% of control animals that did not receive Hsp65 gene gun treatments developed disease within 6 weeks of challenge. The Hsp65 antigen was obtained, prepared, and delivered using techniques as described above in Example 1. In this regard, the nucleic acid sequence and corresponding amino acid sequence for the 65 kDa heat shock protein of *M. tuberculosis* (Hsp65) are known, and are disclosed herein as SEQ ID NOs 1 and 2.

Thus, this is the first demonstration where Hsp65 immunization has resulted in complete protection against CIA in mice. In an earlier report, the immunodominant Hsp65 180-188 epitope (the
5 immunodominant epitope responsible for both disease induction and protection in AA) failed to provide protection against CIA in mice, but was protective against AA (Yang et al., *Clin. Exp. Immunol.* 87:99-104 (1992)). The ability to provide complete protection
10 against CIA in this case suggests that active immunotherapy via epidermal gene transfer has significant potential to modulate existing anti-self inflammatory responses.

To correlate the above-described Hsp65 DNA-mediated protection with antigen-specific immune
15 responses in treated animals, CII specific IgG subclass responses were measured in both the pretreated and control challenge groups; these data are shown in Figure 3. More particularly, serum
20 samples from mice challenged with bovine CII in Freund's adjuvant were collected 42 days after challenge. Half of the animals received two Hsp65 gene gun deliveries to the abdominal epidermis, at 28 and 14 days prior to challenge, respectively.

25 CII-specific IgG subclass responses were measured by standard ELISA techniques using adsorbed bovine CII as the immobilized phase. Although the Hsp65 DNA pretreatment, in theory, would not directly affect the induction of humoral and cellular immune
30 responses to CII during challenge, the cytokine balance in these animals could have been altered, thus affecting the animals' recognition of the CII challenge in an indirect manner. As can be seen in the data of Figure 3, a small but statistically
35 significant reduction in CII-specific IgG1 and IgG2a antibody levels, was seen in treated animals versus

controls, which results are consistent with a shift in the cytokine balance in treated animals.

Example 3

5 Prevention of CIA with a Gene Encoding CII

 The data described in Examples 1 and 2 suggest that peptide antigens, or nucleic acids encoding the same, delivered to the epidermis via intracellular particle delivery, provide an effective
10 means of stimulating anti-inflammatory immune responses. Since rheumatoid arthritis is a disease involving inflammatory autoimmune recognition, active immunotherapeutic approaches based on gene gun-mediated vector delivery to the epidermis are
15 indicated in the prevention of rheumatoid arthritis.

 Protection against CIA development may also be achieved via delivery of a CII expression vector. Comparisons between gene gun delivery of protein- and DNA-based immunogens in murine influenza and hepatitis
20 B immunization models have demonstrated that DNA immunization leads to significantly stronger humoral and cytotoxic T lymphocyte responses.

 In order to optimize such particle mediated delivery parameters, the following studies are carried
25 out. A human type II procollagen cDNA clone is inserted into a clinical grade CMV intron A-based expression construct. A systematic study examining the efficacy of various viral and cellular promoters in a variety of tissues *in vivo* following gene gun-mediated DNA delivery has demonstrated the enhanced
30 effectiveness of the human CMV immediate early promoter in skin. Cheng et al., *Proc. Natl. Acad. Sci. USA* 90:4455-4459 (1993). Thus, the hCMV promoter, with and without its associated intron A
35 sequence, has been the promoter of choice for most DNA immunization experiments.

To optimize the CII vector delivery regimen, a systematic study involving variations in the number and timing of immunizations is carried out. This study is important since earlier studies employing
5 antigen expression vectors from several microbial pathogens have demonstrated dramatic effects on the quality of resulting immune responses, when both the resting period between doses and the total number of doses were varied. Repetition of multiple dosages
10 with short resting periods therebetween likely favors the development of tolerance-type Th2 immune responses.

Gene gun DNA dosages in excess of 0.025 to 0.05 μg per shot generally yield the maximum level of
15 expression obtainable in a given skin delivery site. Therefore, to increase the dosage beyond this point, the number of shots per immunization are increased.

To examine single shot DNA doses ranging from 1.0 μg to 0.001 μg per dose, the treatment
20 protocol includes a group of animals that receive three shots per immunization. The number of treatments and the length of resting periods between treatments are based on results of the above study investigating these variables. The regimen chosen is
25 the one resulting in CII-specific immune responses exhibiting the greatest anti-inflammatory character and protection. The short resting period groups will likely exhibit the most favorable responses.

To attempt to enhance Hsp65 expression, an
30 appropriate CMV intron A vector can be used. In addition, a heterologous signal peptide coding sequence can be inserted into the CMV Hsp65 vector to enhance immunogenicity via secretion.

As discussed above for the human CII vector
35 optimization studies, it is important to determine how the number of Hsp65 DNA doses and the timing between

doses affect Hsp65-specific immune responses prior to challenge, and how the various immunization schedules affect both CII- and Hsp65-specific immune responses following CII/Freund's challenge. Therefore, a
5 systematic evaluation of variables including the number of immunizations (1 to 4), the length of resting periods between immunizations (1 week to 2 months), and the Hsp65 DNA dosage (1.0 to 0.001 μ g) is performed as described above.

10 Endpoints in this study include the incidence of arthritic disease and disease score and CII- and Hsp65-specific IgG isotype profiles, cytokine profiles, and proliferative T cell responses. Immunization protocols that favor the induction of
15 maximum IgG1, TGF-beta, IL-4, and IL-10 production levels may correlate with enhanced protective effects.

Example 4

Immunization of Animals with DNA Coding 20 for Myelin Basic Protein

This example illustrates the effectiveness of gene gun vaccination with a plasmid encoding the gene for myelin basic protein (MBP), an encephalitogen, in preventing the induction of EAE in
25 rats.

The rat MBP gene was cloned in the expression vector pJW4303 as follows: A 599 bp *Bam*HI-*Hinc*II fragment was isolated from pMBP-1 (positions 58-657) (Roach et al., *Cell* 34:799 (1983)). Two
30 synthetic oligonucleotides:

5'-CTAGCATGGCATCACAGAAGAGACCCTCACAGCGACACG
(SEQ ID NO.); and

5'-GATCCGTGTCGCTGTGAGGGTCTCTTCTGTGATGCCATG
(SEQ ID NO.) were annealed and ligated to the
35 fragment to reconstitute the N-terminal coding sequence and introduce a *Nhe*I site at the 5' end.

The product was cloned in frame with TPA leader peptide encoded by the vector pJW4303, prepared by the method of Lu et al., *J. Virol.* 70:3978 (1996), between the *NheI* and the *BamHI* sites of the plasmid.

5 The resulting construct was designated pJWMBP. A protein of 18.5 kD was detected by Western blot analysis, using a rabbit polyclonal antibody specific for rat MBP, in 143B cells transfected with pJWMBP. Closed circular plasmid DNA was purified from

10 transformed DH5a-competent cells by CsCl-ethidium bromide gradient ultracentrifugation using standard protocols as described by Sambrook et al., *Molecular Cloning-A Laboratory Manual*, 2d Ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New

15 York, 1989.

DNA concentration was determined by optical density at 260nm and confirmed by agarose gel analysis comparing standards of known concentration with varying amounts of restriction endonuclease fragments

20 of the plasmid. There was no detectible chromosomal DNA in the plasmid preparations as assessed by gel analysis. After ethanol precipitation, the DNA plasmid was aliquoted and stored at -70°C and when required, was re-precipitated and dissolved in normal

25 saline at 4°C.

The plasmid DNA was attached to gold particles by adding 100 μ l of 0.1 M spermidine (Sigma Chemical Co.) to a 1.5 ml centrifuge tube containing 50 mg of gold powder (0.95 μ m diameter gold powder).

30 The plasmid and gold were precipitated by adding 200 μ l of 2.5 M CaCl_2 solution during vortex mixing and after settling for 10 minutes, the precipitate was washed extensively with absolute ethanol and resuspended in EtOH at 7.0 mg gold/ml. The DNA/gold

35 preparation was then loaded into a gene gun sample cartridge using known techniques, and then delivered

to experimental animals using an electric discharge particle acceleration device as follows.

Specific pathogen-free female Lewis rats (obtained from the Animal Breeding Establishment, The Australian National University) were anesthetized with ethyl ether and their abdomens shaved with electric hair clippers. Animals were placed on their backs and their abdomens gently stretched by extending the fore and hind limbs. A hand held electric discharge Accell particle bombardment device (Auragen, Inc. Middleton, Wis.) was used for gene gun inoculations in a similar procedure to that described by Eisenbraun et al., *DNA Cell Biol.* 12:791 (1993). This procedure was repeated at 3-5 weekly intervals for a total of 5 deliveries according to the protocol shown in Figure 4.

All animals were bled 3-4 weeks after each immunization, as shown in Figure 4 and total Ig, IgG1, and IgG2a antibody levels were determined in the rat sera using the following ELISA method. Flat bottomed, 96 well ELISA plates (Titertek) were coated with 50 μ l of 10 μ g/ml MBP diluted in 0.01M PBS and incubated at 4°C overnight. The plates were washed 3 times in PBST (0.01M PBS, 0.1% TWEEN 20) and blocked with 100 μ l/well of 3% bovine serum albumin (BSA) in PBST for 1 hr at room temperature. After washing with PBST 3 times, 50 μ l of the diluted sera sample and a rat-anti MBP sera diluted in 1% BSA-PBST was transferred to each well. The plates were incubated either overnight at 4°C or 2h at room temperature, washed 6 times and 50 μ l of the appropriate dilution of each Ig antibody was added to each plate and incubated for 1 hr. After washing, 50 μ l of the ABTS peroxidase substrate system (Kirkegaard and Perry Laboratories, Inc.) was added to each well. After 7 minutes, the reaction was stopped by adding 50 μ l of 1% sodium dodecyl sulphate (SDS). Optical density was measured using a microplate reader

at 405 nm with reference to 490 nm. Levels of antibody were determined using an endpoint calculated by measuring three times the mean value of the background optical density and the standard deviation.

- 5 A positive response was considered to be detection of antibody at dilutions greater than those of corresponding control animals.

In some treated rats, MBP-specific antibodies were detected after they had received only three immunizations with pJWMBP, and by the 5th immunization, 8 of 9 rats had seroconverted (see Table 1 below). In rats that responded, circulating anti-MBP antibody titres ranged from 1/160-1/2560. As outlined in Table 1, analysis of the antibody isotype showed that animals immunized with pJWMBP produced primarily IgG1 responses. Only one animal in this group produced any IgG2a, and this was at a low level (titer: 1/80).

This isotypic response is consistent with previous findings by the present inventors that intradermal DNA immunization of autoantigens using a gene gun to deliver genes preferentially stimulates IgG1 responses, while immunization with intramuscular injections promotes IgG2a responses.

25

30

35

Table 1					
Antibody responses of pJWMBP immunized animals challenged with MBP in CFA					
Plasmid	IgG1 anti-MBP antibody titre Days after challenge				
	0	7	20	Ratio of IgG1/IgG2a	Disease Status
pJW MBP	320	960	40,960	64	NC
	<20	<20	20,480	8	C
	640	1,280	81,920	32	NC
	1,280	1,280	81,920	256	NC
	640	1,280	30,720	24	NC
	640	480	40,960	32	NC
	2,560	5,120	163,840	64	NC
	2,560	20,480	327,680	256	NC
	160	10,240	81,920	32	NC
pJW control	<20	40	20,480	8	C
	<20	<20	2,560	1.3	NC
	<20	<20	5,120	2	C
	<20	<20	15,360	12	C
	<20	<20	10,240	5.3	C
	<20	<20	10,240	4	NC
	<20	<20	40,960	5.3	C
	<20	<20	5,120	2	C
Untreated	<20	<20	10,240	4	C
	<20	<20	20,480	8	C
	<20	<20	10,240	2	C
	<20	<20	5,120	4	C

30 C Clinical Disease
NC No Clinical Disease

35 As shown in Tables 1 and 2, pJWMBP protected 8 of 9 animals from the development of autoimmune encephalomyelitis after challenge with MBP in complete Freund's adjuvant. One animal developed symptoms of

EAE. However, this animal was also the one which failed to seroconvert to MBP after gene gun immunization, suggesting that priming had not been successful. Seven out of 9 animals in the pJW control
 5 plasmid immunized group developed clinical symptoms of EAE within 14 days of challenging with MBP in complete Freund's adjuvant. Four out of four animals in the control, non-immunized group, developed EAE.

10

Table 2 Prevention of EAE by immunizing animals with pJW MBP DNA				
Group	Sick/ Total	Mean Maximum Clinical Score	Histological score†	
			Animals Not Exhibiting Clinical Disease	Animals Exhibiting Clinical Disease
Naive Control	4/4	3.2	-	3.8, 1.0, 3.1, 4.7
pJW Control DNA	6/8	2.1	2.5, 4.5	5.2, 4.1, 5.1, 2.3, 5.5, 3.1
20 pJW MBP DNA	1/9	0.3	0, 0, 0, 0, 0.8, 0.13, 0.25, 0.4, 2.5	*2.5

† Lesions counted per section (60 sections examined).

* Animal that failed to seroconvert after immunization.

25

Animals were sacrificed on day 20 and their brains and spinal cords were collected for histological sectioning and staining. Histological sections from the pJW control and nonimmunized control groups showed extensive inflammatory lesions in the
 30 brain and spinal cord, as noted in the histological scores shown in Table 2. Two animals from the pJW control plasmid-immunized group did not develop clinical signs of disease, despite the fact that they were found to have severe CNS inflammation.

35

Histological sections of the pJWMBP-immunized group revealed that 7 of the 8 protected animals showed

either no inflammatory response, or mild lesions in the meninges. One animal from this group, despite not developing clinical disease, exhibited extensive lesions both in the parenchyma and meninges.

5 The results outlined in Table 2 clearly show that the majority of animals (8 of 9) immunized with MBP DNA via gene gun-administration were protected from developing brain and spinal cord lesions and the associated clinical disease symptoms of EAE. The
10 induction of MBP specific IgG1 antibody and the lack of an IgG2a response suggests that gene gun-immunization resulted in a shift toward a Th2 type of response and suppression of a cytotoxic immune response.

15 However, within 7 days of challenge with MBP in complete Freund's adjuvant, there was a significantly greater increase in the level of circulating IgG1 antibody against MBP in animals which had been immunized with pJWMBP and protected against
20 disease compared to animals from either of the control groups. All groups of animals produced IgG1 anti-MBP antibody by day 20, a time when all control animals had recovered from disease, however, significantly higher levels were observed in those animals which
25 were protected from developing EAE. The ratio of IgG1/IgG2a antibody in protected animals given pJWMBP was consistently higher than in the control groups of animals exhibiting clinical EAE. This suggests that gene gun vaccination with pJWMBP desensitized the
30 animals to MBP and decreased the cytotoxic immune response.

35

Example 5Vaccination Against Myelin Oligodendrocyte Glycoprotein

5 This example demonstrates gene gun vaccination with a plasmid encoding the gene for the encephalitogen, myelin oligodendrocyte glycoprotein in preventing the induction of EAE in rats.

10 In an essentially identical manner to that described for the gene encoding myelin basic protein in Example 4, the human myelin oligodendrocyte glycoprotein (MOG) gene (Hilton et al., *J. Neurochem.* 65:309-318 (1995)) is cloned into the expression vector pJW4303. The plasmid DNA is attached to gold particles as described in Example 4. Specific
15 pathogen-free female Lewis rats, as described in Example 4, are given gene gun inoculations with the plasmid containing cDNA encoding the human MOG antigen. This procedure is repeated 3 to 5 times at weekly intervals in a similar protocol to that
20 depicted in Figure 4. Rats vaccinated in this manner are not expected to develop autoimmune encephalomyelitis.

Example 625 Vaccination Against Proteolipid Protein

This example demonstrates gene gun vaccination with a plasmid encoding the gene for the encephalitogen, proteolipid protein (PLP) in preventing the induction of EAE in rats.

30 Using the molecular biology techniques detailed in Example 4 with respect to preparation of the myelin basic protein nucleic acid molecule, the human PLP gene (Nadon et al., *Development* 110:529-537 (1990)) is cloned into the expression vector pJW4303.
35 The plasmid DNA is attached to gold particles as described in Example 4. Specific pathogen-free female

Lewis rats, as described in Example 4 are given gene gun inoculations with the plasmid containing DNA encoding the human MOG antigen. This procedure is repeated 3 to 5 times at weekly intervals for a total of 3-5 deliveries in a similar protocol to that shown in Figure 4. Rats vaccinated in this manner will not develop autoimmune encephalomyelitis.

Example 7

Vaccination with the Entire cDNA Library of Human Oligodendrocytes

This example demonstrates gene gun vaccination with a plasmid encoding the entire cDNA library of human oligodendrocytes in preventing the induction of EAE in rats.

Human oligodendrocytes are isolated and grown in culture according to the method of Zhou et al., *J. Neurosci. Res.* 42:504-515 (1995) and an expression cDNA library is prepared from these cells. Total RNA is prepared from oligodendrocytes by extraction with RNazol, according to the manufacturer's (Tel-Test, Inc.) instructions. Messenger RNA is isolated using the "mRNA purification kit" (Pharmacia, 27-9258-01).

First strand cDNA is synthesized from 2 μ g mRNA, with 100 ng of random hexamer primer, using the "SuperScript Preamplification System for First Strand cDNA Synthesis" (GIBCO BRL, 18089-011). Second strand cDNA is synthesized by a modification of the method of Gubler and Hoffman, *Gene* 25:263-269 (1983). All reagents used in this preparation are from GIBCO BRL. The first strand cDNA reaction mixture (20 μ l) is added to a premix of 16 μ l of 10X *E. coli* ligase buffer, 2.4 μ l 10 mM cNTP mix and H₂O, on ice. RNase H (1.6 U), DNA polymerase I (40 U) and *E. coli* DNA ligase (40 U)

are added and the reaction (final volume, 160 μ l) is incubated for 1 hr at 16°C, then 1 hr at room temperature. Glycogen carrier (10 mg; Boehringer-Mannheim) is added and the cDNA purified by phenol extraction and 2 ether extractions, followed by ethanol precipitation with 2 M ammonium acetate, washed in 70% ethanol and resuspended in 20 ml 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (TE). Double stranded cDNA (1 μ l) is digested with 12 U of Sau3A (New England Biolabs) at 37°C overnight. The restriction enzyme is inactivated at 70°C for 30 min, and the DNA is ethanol precipitated, washed with 70% ethanol and resuspended in 20 μ l TE buffer.

CMV-GH-F1, -F2, or -F3 vectors (20 μ g) are digested with BamHI or BglII according to the method of Durry et al., Nature 377:632-635 (1995) and treated with 2U of calf alkaline phosphatase (30 min at 37°C, 60 min at 50°C, 10 min at 75°C; Boehringer Mannheim). The vectors were extracted with phenol/chloroform, ethanol precipitated and electrophoresed on 1% agarose (low melting temperature, DNA grade agarose, Progen industries) with 0.1 μ g/ml ethidium bromide, excised in the minimum possible volume of agarose and melted at 65°C before adding to the ligation mix. The Sau3A digested cDNA (2 μ l) is ligated to 100 ng of vector (in agarose) with 5U of T4 DNA ligase (Boehringer Mannheim) in 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 10 mM DTT, 0.5 mM ATP in a final volume of 20 μ l, overnight at 16°C.

Electrocompetent *E. coli* are transformed with the ligated cDNA, using a Biorad Gene Pulser (25 μ F, 2.5 kV) with Pulse Controller at 200 Ohms and 0.2 cm cuvettes (according to a protocol from Biorad). The transformation efficiency is approximately 5×10^8 colonies per μ g DNA. 2 μ l Sau3A digested cDNA

(equivalent to 10 ng mRNA) gives from 10 to 25,000 colonies.

The cDNA library is amplified. Thus, after electrotransformation, the library is plated out at high density on L-broth containing 100 µg/ml ampicillin (approximately 30,000 per 9.5 cm square plate). The colonies are grown overnight to a diameter of 0.2-0.3 mm, and rinsed from the plate in a total of 10 ml L-broth per plate. L-broth from several plates is combined, glycerol added to 15% v/v, and the library stored in 1 ml aliquots at -80°C. Separate libraries are generated for each of the 3 CMV-GH vectors.

DNA is prepared in the following manner. L-broth, containing 100 µg/ml ampicillin (70 ml) is inoculated with a 1 ml library aliquot and grown overnight at 37°C with vigorous shaking. The next day, 1 liter baffled flasks containing 500 ml L-broth, containing 100 µg/ml ampicillin are inoculated with 10 ml of overnight culture and grown overnight at 37°C. Closed circular plasmid DNA is purified by CsCl-ethidium bromide gradient ultracentrifugation and attached to gold beads as described in Example 4. Immunization of rats with the Acell particle bombardment device (Auragen, Inc., Middleton, Wis) is used according to the protocol in Example 4.

Accordingly, novel methods for treating or preventing autoimmune disease have been described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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- 15 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
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25 (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4380 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 30 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 252..1874
(D) OTHER INFORMATION: /product= "65 kDa heat
shock protein"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- 35 TCGAACGAGG GCGGTGACCC GGTGCGGGGC TTCTTGCACT CGGCATAGGC GAGTGCTAAG 60
AATAACGTTG GCACTCGCGA CCGGTGAGTG CTAGGTCTGGG ACGGTGAGGC CAGGCCCCGTC 120
GTGCGAGCGA GTGGCAGCGA GGACAACTTG AGCCGTCCGT CGCGGGCACT GCGCCCCGCC 180

	AGCGTAAGTA GCGGGGTTGC CGTCACCCGG TGACCCCGT TTCATCCCG ATCCGAGGA 240																							
	ATCACTTCGC A ATG GCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC 290																							
		Met	Ala	Lys	Thr	Ile	Ala	Tyr	Asp	Glu	Glu	Ala	Arg	Arg										
		1					5				10													
5	GGC	CTC	GAG	CGG	GGC	TTG	AAC	GCC	CTC	GCC	GAT	GCG	GTA	AAG	GTG	ACA								338
	Gly	Leu	Glu	Arg	Gly	Leu	Asn	Ala	Leu	Ala	Asp	Ala	Val	Lys	Val	Thr								
		15					20					25												
	TTG	GGC	CCC	AAG	GGC	CGC	AAC	GTC	GTC	CTG	GAA	AAG	AAG	TGG	GGT	GCC								386
	Leu	Gly	Pro	Lys	Gly	Arg	Asn	Val	Val	Leu	Glu	Lys	Lys	Trp	Gly	Ala								
		30				35					40					45								
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10	Pro	Thr	Ile	Thr	Asn	Asp	Gly	Val	Ser	Ile	Ala	Lys	Glu	Ile	Glu	Leu								
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	GAG	GAT	CCG	TAC	GAG	AAG	ATC	GGC	GCC	GAG	CTG	GTC	AAA	GAG	GTA	GCC								482
	Glu	Asp	Pro	Tyr	Glu	Lys	Ile	Gly	Ala	Glu	Leu	Val	Lys	Glu	Val	Ala								
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	GCC	AAC	CCG	CTC	GGT	CTC	AAA	CGC	GGC	ATC	GAA	AAG	GCC	GTG	GAG	AAG								626
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	Val	Thr	Glu	Thr	Leu	Leu	Lys	Gly	Ala	Lys	Glu	Val	Glu	Thr	Lys	Glu								
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	Gln	Ile	Ala	Ala	Thr	Ala	Ala	Ile	Ser	Ala	Gly	Asp	Gln	Ser	Ile	Gly								
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25	Asp	Leu	Ile	Ala	Glu	Ala	Met	Asp	Lys	Val	Gly	Asn	Glu	Gly	Val	Ile								
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	ACC	GTC	GAG	GAG	TCC	AAC	ACC	TTT	GGG	CTG	CAG	CTC	GAG	CTC	ACC	GAG								818
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	GGT	ATG	CGG	TTC	GAC	AAG	GGC	TAC	ATC	TCG	GGG	TAC	TTC	GTG	ACC	GAC								866
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30		190				195					200					205								
	CCG	GAG	CGT	CAG	GAG	GCG	GTC	CTG	GAG	GAC	CCC	TAC	ATC	CTG	CTG	GTC								914
	Pro	Glu	Arg	Gln	Glu	Ala	Val	Leu	Glu	Asp	Pro	Tyr	Ile	Leu	Leu	Val								
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	Ser	Ser	Lys	Val	Ser	Thr	Val	Lys	Asp	Leu	Leu	Pro	Leu	Leu	Glu	Lys								
				225					230					235										
35	GTC	ATC	GGA	GCC	GGT	AAG	CCG	CTG	CTG	ATC	ATC	GCC	GAG	GAC	GTC	GAG								1010
	Val	Ile	Gly	Ala	Gly	Lys	Pro	Leu	Leu	Ile	Ile	Ala	Glu	Asp	Val	Glu								
			240					245					250											

	GGC	GAG	GCG	CTG	TCC	ACC	CTG	GTC	GTC	AAC	AAG	ATC	CGC	GGC	ACC	TTC	1058
	Gly	Glu	Ala	Leu	Ser	Thr	Leu	Val	Val	Asn	Lys	Ile	Arg	Gly	Thr	Phe	
	255						260					265					
	AAG	TCG	GTG	GCG	GTC	AAG	GCT	CCC	GGC	TTC	GGC	GAC	CGC	CGC	AAG	GCG	1106
	Lys	Ser	Val	Ala	Val	Lys	Ala	Pro	Gly	Phe	Gly	Asp	Arg	Arg	Lys	Ala	
	270					275					280					285	
5	ATG	CTG	CAG	GAT	ATG	GCC	ATT	CTC	ACC	GGT	GGT	CAG	GTG	ATC	AGC	GAA	1154
	Met	Leu	Gln	Asp	Met	Ala	Ile	Leu	Thr	Gly	Gly	Gln	Val	Ile	Ser	Glu	
					290					295					300		
	GAG	GTC	GGC	CTG	ACG	CTG	GAG	AAC	GCC	GAC	CTG	TCG	CTG	CTA	GGC	AAG	1202
	Glu	Val	Gly	Leu	Thr	Leu	Glu	Asn	Ala	Asp	Leu	Ser	Leu	Leu	Gly	Lys	
				305					310					315			
10	GCC	CGC	AAG	GTC	GTG	GTC	ACC	AAG	GAC	GAG	ACC	ACC	ATC	GTG	GAG	GGC	1250
	Ala	Arg	Lys	Val	Val	Val	Thr	Lys	Asp	Glu	Thr	Thr	Ile	Val	Glu	Gly	
			320					325					330				
	GCC	GGT	GAC	ACC	GAC	GCC	ATC	GCC	GGA	CGA	GTG	GCC	CAG	ATC	CGC	CAG	1298
	Ala	Gly	Asp	Thr	Asp	Ala	Ile	Ala	Gly	Arg	Val	Ala	Gln	Ile	Arg	Gln	
		335					340					345					
15	GAG	ATC	GAG	AAC	AGC	GAC	TCC	GAC	TAC	GAC	CGT	GAG	AAG	CTG	CAG	GAG	1346
	Glu	Ile	Glu	Asn	Ser	Asp	Ser	Asp	Tyr	Asp	Arg	Glu	Lys	Leu	Gln	Glu	
	350					355					360					365	
	CGG	CTG	GCC	AAG	CTG	GCC	GGT	GGT	GTC	GCG	GTG	ATC	AAG	GCC	GGT	GCC	1394
	Arg	Leu	Ala	Lys	Leu	Ala	Gly	Gly	Val	Ala	Val	Ile	Lys	Ala	Gly	Ala	
					370					375					380		
	GCC	ACC	GAG	GTC	GAA	CTC	AAG	GAG	CGC	AAG	CAC	CGC	ATC	GAG	GAT	GCG	1442
	Ala	Thr	Glu	Val	Glu	Leu	Lys	Glu	Arg	Lys	His	Arg	Ile	Glu	Asp	Ala	
20				385					390					395			
	GTT	CGC	AAT	GCC	AAG	GCC	GCC	GTC	GAG	GAG	GGC	ATC	GTG	GCC	GGT	GGG	1490
	Val	Arg	Asn	Ala	Lys	Ala	Ala	Val	Glu	Glu	Gly	Ile	Val	Ala	Gly	Gly	
			400					405					410				
	GGT	GTG	ACG	CTG	TTG	CAA	GCG	GCC	CCG	ACC	CTG	GAC	GAG	CTG	AAG	CTC	1538
	Gly	Val	Thr	Leu	Leu	Gln	Ala	Ala	Pro	Thr	Leu	Asp	Glu	Leu	Lys	Leu	
			415				420					425					
25	GAA	GGC	GAC	GAG	GCG	ACC	GGC	GCC	AAC	ATC	GTG	AAG	GTG	GCG	CTG	GAG	1586
	Glu	Gly	Asp	Glu	Ala	Thr	Gly	Ala	Asn	Ile	Val	Lys	Val	Ala	Leu	Glu	
	430					435					440					445	
	GCC	CCG	CTG	AAG	CAG	ATC	GCC	TTC	AAC	TCC	GGG	CTG	GAG	CCG	GGC	GTG	1634
	Ala	Pro	Leu	Lys	Gln	Ile	Ala	Phe	Asn	Ser	Gly	Leu	Glu	Pro	Gly	Val	
					450				455						460		
30	GTG	GCC	GAG	AAG	GTG	CGC	AAC	CTG	CCG	GCT	GGC	CAC	GGA	CTG	AAC	GCT	1682
	Val	Ala	Glu	Lys	Val	Arg	Asn	Leu	Pro	Ala	Gly	His	Gly	Leu	Asn	Ala	
				465				470						475			
	CAG	ACC	GGT	GTC	TAC	GAG	GAT	CTG	CTC	GCT	GCC	GGC	GTT	GCT	GAC	CCG	1730
	Gln	Thr	Gly	Val	Tyr	Glu	Asp	Leu	Leu	Ala	Ala	Gly	Val	Ala	Asp	Pro	
			480					485					490				
35	GTC	AAG	GTG	ACC	CGT	TCG	GCG	CTG	CAG	AAT	GCG	GCG	TCC	ATC	GCG	GGG	1778
	Val	Lys	Val	Thr	Arg	Ser	Ala	Leu	Gln	Asn	Ala	Ala	Ser	Ile	Ala	Gly	
		495					500					505					

CTG TTC CTG ACC ACC GAG GCC GTC GTT GCC GAC AAG CCG GAA AAG GAG 1826
 Leu Phe Leu Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Glu
 510 515 520 525

AAG GCT TCC GTT CCC GGT GGC GGC GAC ATG GGT GGC ATG GAT TTC TGA 1874
 Lys Ala Ser Val Pro Gly Gly Gly Asp Met Gly Gly Met Asp Phe *
 530 535 540

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 GGGAGCTACG GTACCGAGAA CACCACGCAG TCGGTAGGC AACCTTTGGC CGCTGTGGGC 1994
 GAGTCGGGGG CCGCGTCTCG GTGCAGCAGC GCGCGGATGG GTACGACACC GCAGCGGGCG 2054
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 GACGTTGGG TGGACCGCTT TGGTTAGAT CTGCCCGGC ACGACACCGG ATATGGACAC 4334
 CGTCCCGAGG ATGTGGCGAA GTTACGGGCA CCCGCCGACG GAATTC 4380

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 541 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1 5 10 15
 Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
 20 25 30
 25 Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
 35 40 45
 Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
 50 55 60
 Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
 65 70 75 80
 30 Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
 85 90 95
 Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
 100 105 110
 Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Lys Val Thr Glu
 115 120 125
 35 Thr Leu Leu Lys Gly Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ala
 130 135 140

	Ala	Thr	Ala	Ala	Ile	Ser	Ala	Gly	Asp	Gln	Ser	Ile	Gly	Asp	Leu	Ile	
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					165					170						175	
5	Glu	Ser	Asn	Thr	Phe	Gly	Leu	Gln	Leu	Glu	Leu	Thr	Glu	Gly	Met	Arg	
				180					185					190			
	Phe	Asp	Lys	Gly	Tyr	Ile	Ser	Gly	Tyr	Phe	Val	Thr	Asp	Pro	Glu	Arg	
			195					200					205				
	Gln	Glu	Ala	Val	Leu	Glu	Asp	Pro	Tyr	Ile	Leu	Leu	Val	Ser	Ser	Lys	
		210					215					220					
10	Val	Ser	Thr	Val	Lys	Asp	Leu	Leu	Pro	Leu	Leu	Glu	Lys	Val	Ile	Gly	
	225					230					235					240	
	Ala	Gly	Lys	Pro	Leu	Leu	Ile	Ile	Ala	Glu	Asp	Val	Glu	Gly	Glu	Ala	
					245					250					255		
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				260					265					270			
15	Ala	Val	Lys	Ala	Pro	Gly	Phe	Gly	Asp	Arg	Arg	Lys	Ala	Met	Leu	Gln	
			275					280					285				
	Asp	Met	Ala	Ile	Leu	Thr	Gly	Gly	Gln	Val	Ile	Ser	Glu	Glu	Val	Gly	
		290					295					300					
	Leu	Thr	Leu	Glu	Asn	Ala	Asp	Leu	Ser	Leu	Leu	Gly	Lys	Ala	Arg	Lys	
	305					310					315					320	
20	Val	Val	Val	Thr	Lys	Asp	Glu	Thr	Thr	Ile	Val	Glu	Gly	Ala	Gly	Asp	
					325					330					335		
	Thr	Asp	Ala	Ile	Ala	Gly	Arg	Val	Ala	Gln	Ile	Arg	Gln	Glu	Ile	Glu	
				340					345					350			
	Asn	Ser	Asp	Ser	Asp	Tyr	Asp	Arg	Glu	Lys	Leu	Gln	Glu	Arg	Leu	Ala	
			355				360						365				
25	Lys	Leu	Ala	Gly	Gly	Val	Ala	Val	Ile	Lys	Ala	Gly	Ala	Ala	Thr	Glu	
	370						375					380					
	Val	Glu	Leu	Lys	Glu	Arg	Lys	His	Arg	Ile	Glu	Asp	Ala	Val	Arg	Asn	
	385					390					395					400	
	Ala	Lys	Ala	Ala	Val	Glu	Glu	Gly	Ile	Val	Ala	Gly	Gly	Gly	Val	Thr	
					405					410					415		
30	Leu	Leu	Gln	Ala	Ala	Pro	Thr	Leu	Asp	Glu	Leu	Lys	Leu	Glu	Gly	Asp	
				420					425					430			
	Glu	Ala	Thr	Gly	Ala	Asn	Ile	Val	Lys	Val	Ala	Leu	Glu	Ala	Pro	Leu	
				435				440					445				
	Lys	Gln	Ile	Ala	Phe	Asn	Ser	Gly	Leu	Glu	Pro	Gly	Val	Val	Ala	Glu	
		450					455					460					
35	Lys	Val	Arg	Asn	Leu	Pro	Ala	Gly	His	Gly	Leu	Asn	Ala	Gln	Thr	Gly	
	465				470					475						480	
	Val	Tyr	Glu	Asp	Leu	Leu	Ala	Ala	Gly	Val	Ala	Asp	Pro	Val	Lys	Val	
					485					490						495	

Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Gly Leu Phe Leu
500 505 510
Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Glu Lys Ala Ser
515 520 525
Val Pro Gly Gly Gly Asp Met Gly Gly Met Asp Phe *
5 530 535 540

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We claim:

1. A method for treating or preventing an autoimmune disease in a mammal comprising the steps
5 of:

(a) providing a particle coated with an antigen against which an immune response is mounted in the autoimmune disease, wherein the particle is suitable for delivery into a recipient cell, the
10 particle having an average diameter of about 0.5 to about 5 μm and a density sufficient to allow delivery into the recipient cell;

(b) delivering the particle into the recipient cell of the mammal; and

15 (c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

20 2. The method of claim 1, wherein the cytotoxic immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-2, interferon-gamma and tumor necrosis factor.

25 3. The method of claim 1, wherein the desensitizing immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-4,
30 interleukin-5, interleukin-6, and interleukin-10.

4. The method of claim 1, wherein the autoimmune disease is rheumatoid arthritis.

35 5. The method of claim 4, wherein the antigen is selected from the group consisting of

collagen, the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, and epitopes thereof.

6. The method of claim 4, wherein the
5 antigen is collagen, or an epitope thereof.

7. The method of claim 4, wherein the
antigen is the *Mycobacterium tuberculosis* heat shock
protein Mt Hsp65, or an epitope thereof.

10

8. The method of claim 1, wherein the
autoimmune disease is multiple sclerosis.

9. The method of claim 8, wherein the
15 antigen is selected from the group consisting of
myelin basic protein, myelin oligodendrocyte
glycoprotein, proteolipid protein, and epitopes
thereof.

20 10. The method of claim 1, wherein the
particle is comprised of a metal selected from the
group consisting of gold and tungsten.

11. The method of claim 1, wherein the cell
25 is a skin cell.

12. The method of claim 1, wherein the cell
is a mucosal cell.

30 13. A method for treating or preventing an
autoimmune disease in a mammal comprising the steps
of:

(a) providing a nucleic acid construct which
comprises a coding sequence for an antigen against
35 which an immune response is mounted in the autoimmune
disease, operably linked to control elements such that

the coding sequence can be transcribed and translated in a recipient cell;

(b) delivering the nucleic acid construct into the recipient cell of the mammal; and

5 (c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

10 14. The method of claim 13, wherein the nucleic acid construct is coated onto a particle having an average diameter of about 0.5 to about 5 μ m and a density sufficient to allow delivery into the recipient cell, and step (b) entails delivering the
15 coated particle into the recipient cell.

15. The method of claim 13, wherein the cytotoxic immune response is characterized by the secretion from a T-lymphocyte of one or more mediators
20 selected from the group consisting of interleukin-2, interferon-gamma and tumor necrosis factor.

16. The method of claim 13, wherein the desensitizing immune response is characterized by the
25 secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-4, interleukin-5, interleukin-6, and interleukin-10.

17. The method of claim 13, wherein the
30 autoimmune disease is rheumatoid arthritis.

18. The method of claim 17, wherein the coding sequence encodes an antigen selected from the group consisting of collagen, the *Mycobacterium*
35 *tuberculosis* heat shock protein Mt Hsp65, and epitopes thereof.

19. The method of claim 17, wherein the coding sequence encodes collagen or an epitope thereof.

5 20. The method of claim 17, wherein the coding sequence encodes the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65 or an epitope thereof.

10 21. The method of claim 13, wherein the autoimmune disease is multiple sclerosis.

15 22. The method of claim 21, wherein the coding sequence encodes an antigen selected from the group consisting of myelin basic protein, myelin oligodendrocyte protein, proteolipid protein, and epitopes thereof.

20 23. The method of claim 21, wherein the coding sequence encodes the entire cDNA library of human oligodendrocytes.

25 24. The method of claim 21, wherein the nucleic acid construct comprises an oligodendrocyte gene library comprising one or more coding sequences operably linked to control elements such that the coding sequences can be transcribed and translated in a recipient cell.

30 25. The method of claim 13, wherein the cell is a skin cell.

26. The method of claim 13, wherein the cell is a mucosal cell.

35 27. Use, in the manufacture of a medicament for treatment or prevention of an autoimmune disease

in an animal, of a particle coated with an antigen
against which an immune response is mounted in the
autoimmune disease, wherein the particle is suitable
for delivery into a recipient cell, the particle
5 having an average diameter of about 0.5 to about 5 μ m
and a density sufficient to allow delivery into the
recipient cell.

28. Use, in the manufacture of a medicament
10 for treatment or prevention of an autoimmune disease
in an animal, of a nucleic acid construct which
comprises a coding sequence for an antigen against
which an immune response is mounted in the autoimmune
disease, operably linked to control elements such that
15 the coding sequence can be transcribed and translated
in a recipient cell.

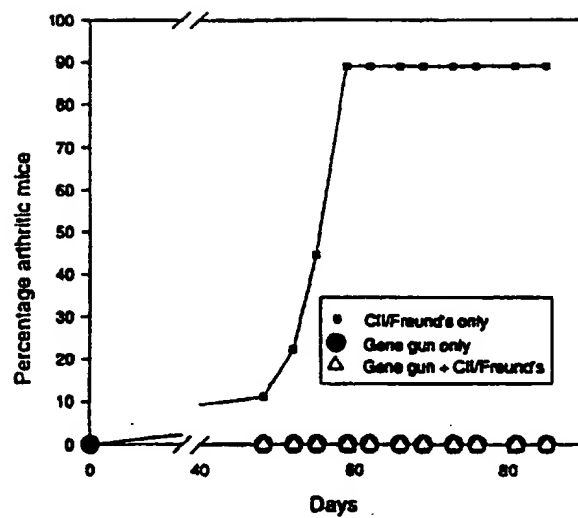
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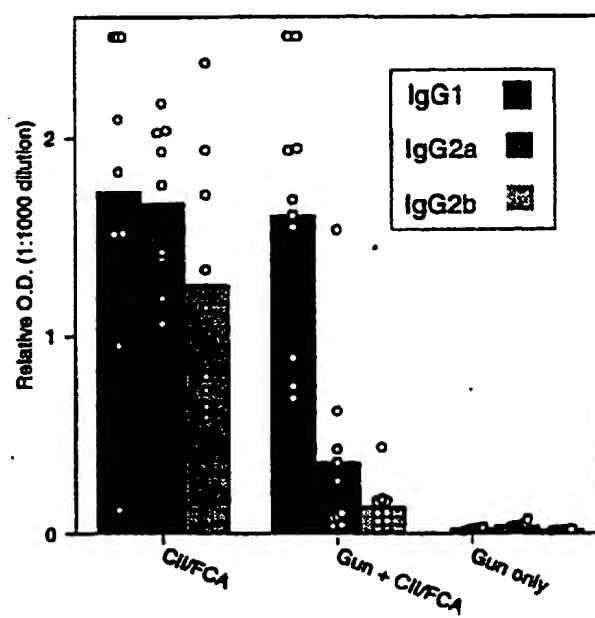
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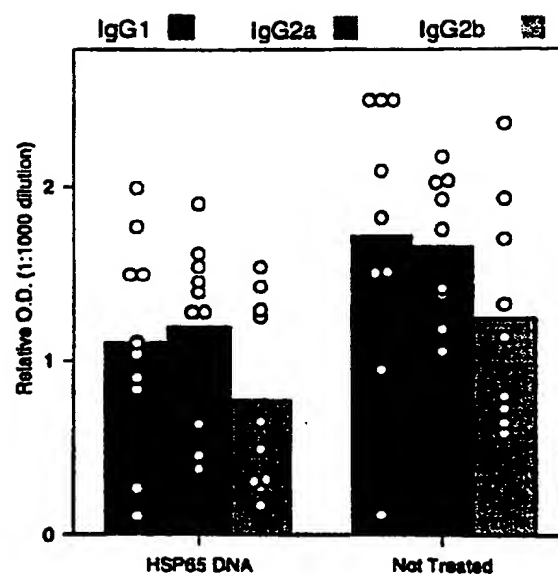
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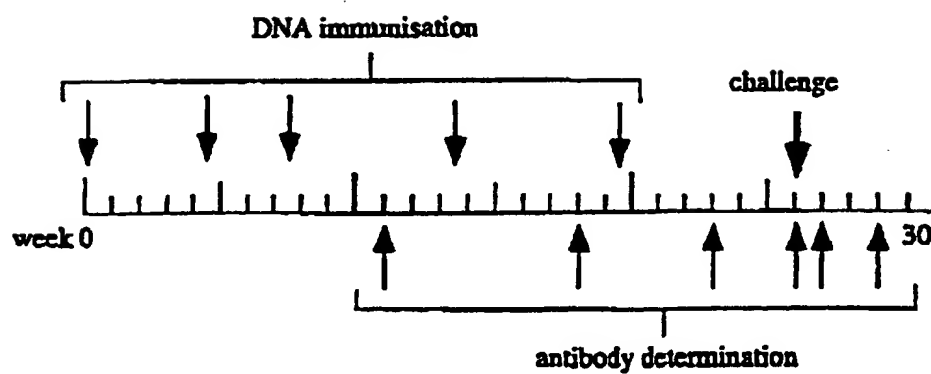
**FIG. 1**

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**FIG. 2**

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**FIG. 3**

**FIG. 4**